

CLAIMS

1. A fluorescent protein derived from Green Fluorescent Protein (GFP) or any functional GFP analogue, wherein the amino acid in position 1 preceding the chromophore has been mutated and wherein the Glutamic acid in position 222
5 has been mutated said mutated GFP has an excitation maximum at a higher wavelength and the fluorescence is increased when the mutated GFP is expressed in cells incubated at a temperature of 30°C or above compared to wild-type GFP.
2. A fluorescent protein according claim 1, wherein the chromophore is in position
10 65-57 of the predicted primary amino acid sequence of GFP.
3. A fluorescent protein according to claim 1 or 2, said protein being derived from *Aequoria victorea* or *Renilla*.
4. A fluorescent protein according to claim 1, wherein the amino acid F in position
64 of the GFP has been substituted by an aliphatic amino acid.
- 15 5. A fluorescent protein according to claim 1, wherein the amino acid F in position
64 of the GFP has been substituted by an amino acid selected from the group
consisting of L, I, V, A and G.
6. A fluorescent protein according to claim 1, wherein the amino acid F in position
64 of the GFP has been substituted by L.
- 20 7. A fluorescent protein according to claim 1, wherein the amino acid E in position
222 of the GFP has been substituted by an amino acid selected from the group
consisting of G, A, V, L, I, F, S, T, N, and Q.
8. A fluorescent protein according to claim 1, wherein the amino acid E in position
222 of the GFP has been substituted by G.

9. A fluorescent protein according to claim 1 having the amino acid sequence disclosed in SEQ ID NO: 4.

10. A fluorescent protein according to claim 1 having the amino acid sequence disclosed in SEQ ID NO: 8.

5 11. A fusion compound comprising a fluorescent protein (GFP) according to claim 1, wherein the GFP is linked to a polypeptide.

12. A fusion compound according to claim 11, wherein the polypeptide is a kinase, preferably the catalytic subunit of protein kinase A, or protein kinase C, or Erk1, or a cytoskeletal element.

10 13. A nucleotide sequence coding for the fluorescent protein of claim 1.

14. A nucleotide sequence according to claim 13, shown in SEQ ID NO: 3.

15. A nucleotide sequence according to claim 14, shown in SEQ ID NO: 7.

16. A nucleotide sequence according to claim 13 in the form of a DNA sequence.

17. A host transformed with a DNA construct according to any one of claims 13-
15 16.

18. A process for preparing a polypeptide, comprising cultivating a host according to claim 17 and obtaining therefrom the polypeptide expressed by said nucleotide sequence.

19. A method for measuring the protein kinase activity, dephosphorylation activity
20 or protein distribution in an *in vitro* assay comprised of transforming a host cell with a DNA construct according to claim 13 and measuring the fluorescence of cells transformed with the DNA construct.